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# Brain Tumors in Man and Animals: Report of a Workshop*

**This report summarizes the results of a workshop on brain tumors in man and animals. Animals, especially rodents are often used as surrogates for man to detect chemicals that have the potential to induce brain tumors in man. Therefore, the workshop was focused mainly on brain tumors in the F344 rat and B6C3F1 mouse because of the frequent use of these strains in long-term carcinogenesis studies. Over 100 brain tumors in F344 rats and more than 50 brain tumors in B6C3F1 mice were reviewed and compared to tumors found in man and domestic or companion animals. In the F344 rat, spontaneous brain tumors are uncommon, most are of glial origin, and the highly undifferentiated glioblastoma multiforme, a frequent tumor of man was not found. In the B6C3F1 mouse, brain tumors are exceedingly rare. Lipomas of the choroid plexus and meningiomas together account for more than 50% of the tumors found. Both rodent strains examined have low background rates and very little variability between control groups.**

## Introduction

Cancer of the central nervous system is of interest to toxicologists because of its reported association with various occupations in man. Rodents are often used as models for detecting possible environmental carcinogens, yet systematic consideration of brain pathology in long-term rodent carcinogenicity did not receive the attention it deserved. It was this aspect that prompted the National Toxicology Program to convene a conference on brain tumors on September 5 and 6, 1984, in Research Triangle Park, NC. The conference was focused on comparative aspects of human and animal brain tumors and on newer techniques that can be used as diagnostic aids for detecting and classifying brain tumors. The main themes of the conference were: normal structure of the brain and aids to anatomic pathology of brain lesions; incidences, clinical diagnosis, and morphological classification of brain tumors in man and animals; and experimentally induced brain tumors and *in vitro* studies.

Experts in human and veterinary neuropathology presented the state-of-the-art in these different areas. The conference was attended by approximately 150 governmental and nongovernmental scientists.

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## Incidence of Brain Tumors in Man and Animals

### Man

Descriptive epidemiologic studies of primary nervous system neoplasms in well defined human populations have demonstrated that despite our diagnostic sophistication, current incidence rates probably underestimate the magnitude of this health problem. Despite variations among the different data resources in reporting and diagnostic practices, a general pattern of age-specific incidence rates has emerged: a small peak in childhood, followed by a higher peak reaching a maximum between ages 60 and 80 and then declining after those ages. One notable exception to the pattern is provided by tabulations for Rochester, Minnesota, where, after a small childhood peak, there is a sustained increase in the incidence rate with increasing age. The relatively large proportion of cases first diagnosed at autopsy in Rochester accounts, in large part, for the different age-specific incidence curves, particularly among the elderly. The results of the Rochester, Minnesota, investigations suggest that substantial numbers of asymptomatic tumors are never diagnosed in older individuals and if autopsy rates increase, the age-specific pattern of incidence rates will more closely resemble the Rochester curve.

The various histologic types of brain tumors reveal sufficiently distinct epidemiologic patterns to be classified as separate diseases. It may be misleading to consider primary intracranial neoplasms as a single group, since the overall picture simply reflects these distinctive individual patterns and the relative frequency of diag-

nosed cases of these histologically separate neoplasms in the population under study. In children, the relative frequencies of these various tumors differ markedly from their frequencies among adults. For example, while medulloblastoma is the most commonly occurring brain tumor among children, it is relatively rare in adults. Astrocytoma is second in children, whereas it ranks third in frequency among adults. Glioblastoma multiforme, ranking third in children, accounts for more than half of the histologically confirmed intracranial tumors among adults. Pituitary chromophobe adenoma, while common among adults, is observed much less often in children. Meningioma, which ranks second in adults, is relatively infrequent in childhood.

The rare familial aggregation of certain primary intracranial neoplasms and their association with conditions demonstrating a defined pattern of inheritance identify the high-risk patient. Such identification is of immediate clinical value in alerting the physician to the likely presence of these tumors. Recent analytic epidemiologic investigations have focused on potential etiologic factors within the occupational environment, whereas others have utilized a more general exploratory approach in searching for such factors. It is hoped that the more detailed evaluation of patients at high risk for brain tumors will lead to a better understanding of the mechanisms involved in oncogenesis within the nervous system.

## Domestic Animals

With the exception of a few sporadic reports, little is known about brain tumors in most animal species. Only domestic animals and laboratory animals have been sufficiently studied to evaluate frequency and spectrum of tumors of the nervous system. Published estimates indicate that the frequency of brain tumors in all domestic animals combined is low, whereas intracranial neoplasms are a relatively common finding in dogs (*1–3*). The incidence rate (number of cases per 100,000 of the population at risk) of nervous system tumors has been estimated in dogs and cats to be 14.5 and 3.5, respectively.

The frequency of various tumor types in specific domestic animal species was estimated on the basis of our own material and analysis of the literature since 1968. A total of 1412 intracranial tumors (including metastatic and pituitary growths) were found which were distributed among the various species as follows: 984 dogs, 216 cats, 68 bovines, 20 horses, 12 sheep, and 10 pigs. Thus, brain tumors are much more common in dogs than in other species, although—at least in our own material—the number of dogs necropsied is not greater than that of food animals. The low number of brain tumors in horses in this series is in part related to the small overall number of horses examined but is also consistent with old publications in which large numbers of horses with neurologic disease were studied. The apparent low incidence in food animals is certainly related to their very low life expectancy.

Only 20 tumors were available in the horse with 12 primary brain tumors consisting of 3 neuroectodermal and 9 mesenchymal neoplasms. Of the 68 bovine brain tumors surveyed in cattle, 26 were neuroectodermal, including 6 medulloblastomas, and 34 were primary mesenchymal growths including 20 meningiomas. In the other food animal species (sheep, goat, pig) only 3 neuroectodermal and 10 mesenchymal tumors were found. Of the 216 brain tumors surveyed in cats, only 10 neuroectodermal but 148 mesenchymal tumors including 117 meningiomas were found.

The dog has not only the highest frequency of intracranial neoplasms but also the broadest spectrum of types many of which are very similar to human brain tumors. There is a higher incidence among certain breeds, such as boxers, Boston terriers and bulldogs. Primary neuroectodermal and mesenchymal tumors appear to occur with similar frequency. Meningiomas and sarcomas are well represented and are rather ill-defined. Some of these latter lesions are histiocytic lymphosarcomas, whereas others may be true histiocytic tumors or even granulomas.

The canine neuroectodermal tumors in our survey consisted of 92 astrocytomas, 99 oligodendrogliomas, 42 choroid plexus papillomas, 40 glioblastomas, 20 medulloblastomas, 19 ependymomas, 4 gliomatosis, 1 pinealoma, and 24 unclassified gliomas. The results of immunocytochemical staining for the glial fibrillary acidic protein (GFAP) in 74 canine neuroectodermal tumors confirmed that many canine glioma types are very similar to human brain tumors but also indicated a higher proportion of undifferentiated gliomas in dogs as compared to man.

## Laboratory Animals

A wide variety of animal species are used in biological and clinical research. The choice of the animal species is dependent on the type of research carried out and varies from primates to protozoa. This overview is confined to those animal species that are most commonly used in biomedical and toxicological studies. These are in decreasing order of frequency: rats, mice, Syrian hamsters, dogs, rabbits, monkeys, guinea pigs, and cats. Brain tumors in dogs and cats are discussed above.

A review of the literature on the occurrence of brain neoplasms in the above-mentioned animal species reveals a variety of incidence within and among the species. Reasons accounting for these variations are: differences in ages of the animals in the studies, differences in breeds, strains, substrains and stocks studied, and differences in the extent in which brain tissue is examined.

The most valuable information on the occurrence of brain tumors in various animal species comes from studies in which animals are allowed to live out their lifespan. Since nearly all lifetime studies are confined to mice and rats, the incidences in these two animal species are probably the most reliable figures available from all animal species studied so far.

**Table 1. Brain tumor incidence in various mouse strains and hybrids.[a]**

| Strain | No. of mice studied | Incidence, % |
|---|---|---|
| VM | 9,980 | 1.7 |
| BRVR | 2,640 | 1.1 |
| C57BL | 11,900 | 0.02 |
| C57BL/6J | 817 | 0.12 |
| BALB/c | 4,278 | 0.09 |
| BALB/c | 2,560 | 0.04 |
| B6C3F1 | 5,065 | 0.08 |
| B6C3F2 | 334 | 0.29 |

[a] Modified from Swenberg (*4*).

Brain neoplasms in mice are exceedingly rare with exception of two strains and their crosses, the VM and BRVR (*4*). The respective incidences in these two strains are 1.6 and 1.1%. Astrocytomas constitute nearly 100 percent of the intracranial neoplasms in these two strains. In all other mouse strains studied so far, the incidence does not exceed 0.3% (Table 1). If one excludes the two mouse strains and their crosses that have an unusually high occurrence of brain tumors, the overall incidence in mice is less than 0.01%.

Brain tumors in rats are much more common than in mice. Data derived from lifetime studies show incidences up to 7.1% (Wistar AF/Han-EMD strain) (Table 2). The F344 rat which is widely used in carcinogenesis testing programs in the U. S. and abroad shows lifetime incidences of 2.9 and 2.2% in males and females, respectively. The incidence at 2-year of age is 1.1% in both sexes, but the incidence varies from 0 to 4% in control groups of 50 animals each.

As mice, the incidence of brain neoplasms in Syrian hamsters is exceedingly rare. As far as is known, no reports on spontaneous brain tumors have been published in this animal species. Lifetime data of the National Toxicology Program show two astrocytomas in 457 male (=0.4%) and none in 463 female Syrian hamsters examined.

Neuroepithelial tumors have not been reported in rabbits and guinea pigs and only rarely in monkeys. Since lifetime studies dealing with these animal species are lacking, the paucity of brain neoplasms in these species does not necessarily mean that they are more resistant to the development of brain neoplasms than other species.

**Table 2. Brain tumor incidence and most common tumor type in various rat strains: lifetime studies.**

| Strain | Incidence (%) in Males | Incidence (%) in Females | Most common tumor type | Reference |
|---|---|---|---|---|
| WAG/RIJ | 2.4 | 3.9 | Astrocytoma | Burek, 1978 (*48*) |
| AF/Han-EMD | 7.1 | 3.3 | Granular cell tumor | Sumi et al., 1976 (*49*) |
| ACI/seg HapBR | 2.7 | NS[a] | Granular cell tumor | Ward et al., 1983 (*29*) |
| BN/BIRIJ | 2.7 | 5.9 | Granular cell tumor | Burek, 1978 (*48*) |
| Osborne-Mendel | NS[a] | 1.5 | Ependymoma | Dagle et al., 1979 (*50*) |
| Sprague-Dawley | NS[a] | 1.3 | Astrocytoma | Dagle et al., 1979 (*50*) |
| F344 | 0.0 | NS[a] | | Coleman et al., 1977 (*51*) |
| F344/N | 2.9 | 2.2 | Astrocytoma | Solleveld et al., 1984 (*52*) |

[a] NS = not studied.

## Clinical Diagnosis of Brain Tumors in Man and Animals

### Man

Although methods of diagnosis of primary brain neoplasms in humans have become increasingly sophisticated, the first step in making such a diagnosis remains the alert clinician's suspicion of the problem. Appropriate studies can then be used to examine particular areas of the nervous system. The clinician must be sensitive both to worrisome signs and symptoms and to the epidemiology of these conditions. For example, lateralized headaches in a 50-year-old woman should make one concerned about a supratentorial mass, such as a meningioma or high-grade glioma, whereas headaches and vomiting in a 10- year-old boy should lead one to suspect a posterior fossa mass, such as a medulloblastoma.

Two general categories of signs and symptoms may be present in patients with intracranial mass lesions. Typical symptoms produced solely by the mass and consequent increased intracranial pressure are headache (often worse in the recumbent position) and confusion. Signs include papilledema and altered mental status (such as lethargy, stupor, or coma). Vomiting and hiccoughs are also occasionally present. The other general category of symptomatology in these patients includes those findings that reflect dysfunction of particular areas of the brain, such as hemiparesis, hemianesthesia, visual field loss, aphasia, and focal seizures. The more circumscribed the neurologic abnormality, e.g., a particular form of aphasia or weakness confined to one extremity, the more precisely one can localize the pathologic process.

However, in this era, clinical suspicion and diagnostic acumen merely aid in the choice and technique of imaging studies. These include radiographic, or X-ray, studies as well as a variety of highly sensitive nonradiographic imaging techniques. Radiographic methods include skull X-ray, angiography, pneumoencephalography, cisternography with positive-contrast media, and finally, computerized tomography (CT). Many of these tests are now virtually obsolete with the advent of CT and nuclear magnetic resonance (NMR) imaging. Nevertheless, all have been useful and many are still important in certain situations. The most valuable imaging technique that is widely available for diagnosing brain tumors is CT. Its sensitivity varies with both the nature and size of the lesion. Primary brain tumors are usually hypodense in comparison to normal brain, and they show variable enhancement after intravenous in-

fusion of a contrast agent. The degree of enhancement reflects disruption of the blood brain barrier and to some extent correlates with the degree of anaplasia. Central low-density, nonenhancing areas within high-grade gliomas reflect necrotic tissue (*5*). The full potential of NMR in the diagnosis of primary brain tumors is uncertain. This nonradiographic imaging technique utilizes differential magnetic relaxation properties of different tissues (*6*). It is apparent that it is more sensitive than CT in detecting small or subtle lesions. It is also more useful in areas that are difficult to visualize by CT such as the posterior fossa. Specificity of the abnormalities on NMR remains to be determined.

Although imaging techniques have become highly elegant and sophisticated, it remains true that the pathologic specimen is the *sine qua non* of tumor diagnosis. Without tissue the diagnosis is speculative, and in difficult cases the burden of proof should reside with the physician who wishes to treat without tissue. The pathologic specimen is usually obtained by open and wide resection of the lesion. However, when resection is not advisable, needle biopsy under stereotactic control is feasible, safe, and usually provides adequate material for diagnosis. Although tissue sampling is necessarily limited and relief of symptoms cannot be anticipated, needle biopsy is an important advance since it provides material for accurate diagnosis and prognosis.

## Domestic Animals

Primary brain tumors occur in all the domestic animals but the dog and cat are those studied most carefully. Primary brain tumors in these animals comprise nearly 4% of admissions at institutional urban veterinary neurology services. Until CT became available, the history, neurologic examination, spinal fluid examination, plain radiography, electroencephalography, arteriography, and pneumoencephalography were the tools in tumor localization. These methods could determine the rostro-caudal and medio-lateral neuraxial position of tumors with moderate accuracy, but prosencephalic masses were often localized with insufficient precision for surgical intervention.

The CT is now widely accepted as the tool of choice for imaging intracranial tumors in dogs and cats and the first reports of individual cases and case series have appeared in the recent clinical literature. Meningiomas, a variety of astrocytomas, metastatic tumors and pituitary tumors have been studied in plain and contrast-enhanced CTs. These studies also allow assessment of accompanying cerebral edema, midline shift, ventricular compression or hydrocephalus. Variations in the appearance of tumors on CT parallel these reported for humans.

This technology has resulted in such an improvement in the precision of tumor localization that biopsy and excision are now common in the larger services. This has resulted in improved clinical care and the availability of neoplastic tissue for further study.

## Laboratory Animals

NMR imaging is one of the most exciting and rapidly developing fields in diagnostic medicine (*6–8*). In 1946, the principles of magnetic resonance for liquids and solids were independently described by Bloch and Purcell (*9–11*). These efforts earned each a Nobel prize in 1952. In 1973, Lauterbur (*12*) published the first two-dimensional, proton NMR image of a container of water. In the few years since, prototype imaging devices that were developed for use with laboratory animals have been replaced by whole body NMR imaging systems. Clinical trials have been conducted evaluating the potential of NMR imaging as an independent technique or comparing it with the established technique of X-ray CT. Because of the accumulated knowledge concerning the acquisition and interpretation of CT scans of the brain, this organ has often been used for evaluation of NMR and CT capabilities (*13–16*).

NMR imaging does not use ionizing radiation to produce the cross-sectional pictures. Instead, it relies upon the inherent property of those nuclei with a combination of an odd number of protons and neutrons to possess a net angular momentum or spin. Charged particles with linear velocity generate a magnetic field that is perpendicular to the direction of movement. Similarly, nuclei with net rotation or spin have charge (protons) and angular velocity. They behave, therefore, as small rotating magnets with the magnetic field being perpendicular to the angle of rotation. Among those elements in the body susceptible to NMR probing, the hydrogen nucleus (proton) is the most abundant (body by weight, 60–90% water) and has the highest degree of NMR sensitivity. Normally, protons are randomly arranged and there is no net magnetization in the tissue. If placed in a static, external magnetic field, however, protons align parallel (low energy state) or antiparallel (high energy state) to the axis of the static magnetic field and consequently a macroscopic magnetization vector is produced in the tissue. The protons do not align exactly with the static field, but are at a slight angle to the axis about which they rotate or precess. The frequency of precession is directly related to the strength of the external magnetic field. This frequency, which is in the radiowave range, is the source of the signal used for generation of the NMR images. By applying a radiofrequency (RF) pulse which matches the precessional frequency of the nuclei and which rotates perpendicularly to the static field, the macroscopic magnetic vector can be tilted from its original plane and acquire a transverse component. When the RF signal is turned off, the nuclei spin briefly in the altered plane and then return to the previous alignment in the static field. An oscillating voltage (induced by the transverse magnetization vector) is produced in a receiver coil located in the transverse plane. The frequency of this "signal" is the same as the precessional frequency of the protons.

The realignment process is characterized by two time constants, T1 and T2. T1, or the spin-lattice relaxation time, is a measurement of the time required for the

nuclei to return to longitudinal alignment in the static field at the end of a transverse RF pulse. T2, or the spin-spin relaxation time, reflects the time required for disappearance of the transverse component of the magnetic moments of the nuclei during the realignment process. T1 is influenced by the interaction of the precessing nuclei with the surroundings of lattice while T2 is influenced by the homogeneity of the static magnetic field and by interactions between precessing nuclei. Because of these relationships, T1 values for pure water or for water containing other small molecules are long and those for water containing large molecules (such as tissue fluids with proteins, fats and complex carbohydrates) are relatively short. T2 values are longer for liquids than they are for solids, and with liquids, the values decrease as larger molecules are added. In a T1-weighted NMR image of the brain, subcutaneous fat appears bright (short T1), and white matter, grey matter, and cerebrospinal fluid (CSF) appear progressively darker (increasing T1 values). Generally, the NMR signal becomes stronger (image becomes brighter) with an increase in magnetic field strength, an increase in proton density, a decrease in T1, an increase in T2, and a decrease in motion (flow).

Different NMR imaging techniques can be used such that combinations of proton density, T1, T2, and flow characteristics can be emphasized. In the production of NMR images of the brain, those techniques that measure proton density and T1 provide excellent grey-white matter contrast and good anatomical detail. T2-weighted images of the brain have less contrast than T1-weighted images. Various pathologic processes in the brain have been evaluated by NMR imaging. These include infection, infarction, hemorrhage, degenerative diseases, psychological disorders, and neoplasms. Concerning the latter, some generalizations can be made. NMR images are often equal to or better than CT scans at detecting and delineating a neoplasm. Detection with CT scans depends upon differences in tissue density. NMR images incorporate differences in proton density and alterations in tissue water or fat composition produced by a disease process. NMR imaging techniques that emphasize T1 and T2 values usually permit the distinction of a neoplasm from normal host tissue. Generally, T1 values are increased for neoplasms (darker on image) and malignant tumors often have longer T1 values than benign ones. Overlapping values between tumor types occur too frequently, however, to permit any histologic classification based only on imaging information. Also, T1 and T2 values for tumors are not unique and can overlap those for other types of lesions. Additional data must be considered before a presumptive diagnosis of neoplasia can be made. The scientific application of NMR imaging for the detection of brain tumors in laboratory animals is the same as that for human beings. For animals such as the rat or mouse, the limiting factor is the size of the brain and the resulting difficulty in resolution. For larger animals (e.g., dogs, cats, primates) brain size is not a limiting factor. With the production of stronger magnets with more homogenous fields, NMR imaging of the brain of small laboratory animals should become a viable research tool.

# Normal Structure of Cellular Elements of the Brain

## Supporting Cells

The cellular elements of the central nervous system consist of the neurons and a variety of supporting cells. The present account will focus on the supporting cells, since these are the elements most commonly involved in the formation of tumors in the brain.

***Neuroglial Cells.*** The neuroglial cells are generally considered to be of three main types, astrocytes, oligodendrocytes, and microglial cells.

Astrocytes occur throughout the brain substance and are of two varieties, protoplasmic and fibrous. Protoplasmic astrocytes are present in grey matter and fibrous astrocytes in white matter. Both have a pale cytoplasm and apart from location, one difference between them is that fibrous astrocytes contain more 8 to 9 nm filaments than the protoplasmic ones. Both varieties have processes which pass between the neuronal elements and some of these processes end in expansions which form a glial limiting membrane on the outside of the brain and around penetrating blood vessels. Gap junctions are common between astrocytes and the number of gap junctions seems to be reduced between astrocytes involved in the formation of tumors. Astrocytes readily become edematous and can act as phagocytes.

Oligodendrocytes occur in both white and grey matter. They are more common in white matter since their principal function is to form myelin sheaths. Each oligodendrocyte forms a number of internodal lengths of myelin, the sheaths being produced at the ends of the processes extending from the cell body.

Microglial cells are small cells with an electron microscopic appearance similar to that of oligodendroglia. During aging microglial cells accumulate lipofuscin and they respond to disruptive events such as trauma, lesions, and inflammation by dividing and becoming phagocytic. There is evidence to suggest that when blood vessels are disrupted monocytes may enter the brain and assume a morphology similar to that of the microglial cells which normally inhabit the brain.

***Ependymal Cells.*** The ventricles of the brain are lined by an epithelium, the ependyma. The cells form a single layer and are ciliated, producing movements of the cerebrospinal fluid. Adjacent ependymal cells are joined by gap junctions and zonulae adherences. Cerebrospinal fluid can pass between ependymal cells to provide the interstitial fluid of the brain substance. In some areas, the cells beneath the ependyma, the subependymal cells remain, mitotically active and can generate neuroglial cells postnatally.

***Choroid Plexus.*** The choroid plexus forms a secreting epithelium which is derived from the ependyma. However, the choroidal cells have few cilia and instead are covered by microvilli. The function of the choroid

plexus is to secrete cerebrospinal fluid. To ensure that fluid passes through and not between cells, adjacent choroidal cells are joined together by tight junctions at their ventricular ends. The blood vessels in the stroma of the choroid plexus are unusual, for they are fenestrated. It is of interest that free macrophages, the Kolmer cells, lie on the ventricular surface of the choroid plexus.

# Aids to the Anatomic Pathology of Brain Tumors

## Electron Microscopy

Since electron microscopy can resolve difficult diagnostic and taxonomic problems, it is a potentially valuable tool in the diagnosis of natural and experimental brain tumors. As currently applied, the utility of this technique depends largely on its ability to resolve subcellular organelles of diagnostic interest. The presence and specificity of these organelles, however, varies from lesion to lesion and from cell to cell. On one hand are those, such as synapses, premelanosomes, and neurosecretory granules that are specific for a certain type of cell and can establish, by themselves, a pathologic diagnosis. At the other extreme are the undifferentiated lesions which lack specific ultrastructural features which unfortunately are more frequent. Intermediate are the common neoplasms with organelles such as cell junctions, cytoplasmic filaments, microtubules, microvilli, and cilia which, although not specific for a certain cell type, are helpful when considered with the light microscopic features and location of the lesion.

To assess the contribution of electron microscopy to the diagnosis of central nervous system neoplasia, surgical specimens previously studied ultrastructurally were reviewed for a 5-year period. During this time, 59 cases had been examined; 30 had been studied by electron microscopy primarily for teaching purposes and 29 were studied as part of the formal diagnostic workup. For each case, the usefulness of electron microscopy was estimated. This was done in a semiquantitative fashion, assigning 0 for cases in which electron microscopy provided no additional diagnostic information, to 4+ when electron microscopy provided a definitive diagnosis. A 1+ was assigned when the lesion provided some additional information to the light microscopy, and often served to reinforce the light microscopic diagnosis. A 2+ was given when electron microscopy obtained significant information in addition to the light microscopic findings. A 3+ was given when the electron microscopy added more significant information to the light microscopic findings, and the two techniques in combination permitted a definitive diagnosis.

Of the 29 cases studied as part of the formal diagnosis, electron microscopy was not felt to make a significant contribution in 16. Of the remaining 13 cases, there was one, a small cell neoplasm of the filum terminale with neurosecretory granules in which the electron microscopic findings established a neuroendocrine neoplasm. This lesion was given a 4+. Two lesions were assigned to 3+. These were a papillary meningioma in which the classic ultrastructural features of meningioma was apparent, and a nerve sheath myxoma of the vagus nerve in a 9-year-old where the multiple basement membranes were a significant finding. Two lesions, a meningioma and a choroid plexus carcinoma, were assigned 2+. The remaining seven lesions were given a 1+ in light of the small contribution made by electron microscopy.

It is concluded from the above that electron microscopy as applied to human tumors can be a valuable technique and make a significant contribution to pathologic diagnosis. However, if the present cases are representative, its contribution will frequently be limited. This, when considered with the necessary time and expense, suggests that electron microscopy be used judiciously in the diagnosis of human brain tumors.

## Immunohistochemistry

Immunohistochemistry is a useful technique for the demonstration of a specific antigen in fixed or frozen tissue. It requires a polyvalent antiserum or a monoclonal antibody prepared against the antigen of interest. Immunofluorescence is generally used for frozen tissue, and immunoperoxidase (PAP method) for formalin-fixed, paraffin-embedded tissue. This technique can be useful in the diagnosis of brain tumors provided the specificity of the antiserum or monoclonal antibody is well characterized. Structural antigens, such as the intermediate filament (IF) proteins, are generally comparably expressed in normal and neoplastic cells of the same origin. Other antigens, particularly metabolic markers, may have a different distribution in neoplastic cells from that observed in their normal counterparts, and caution must therefore be exercised in the interpretation of the tumors expressing these markers.

The most important and reliable markers for the diagnosis of brain tumors appear to be the IF proteins. The neurofilament proteins (NF) are expressed only in tumors of putative neuronal origin or with presumed neuronal differentiation (*17*). They are often not expressed in poorly differentiated malignant tumors assumed to be of neuronal origin, such as central neuroblastoma, medulloblastoma, and pineoblastoma. They are easily demonstrable in the ganglion cell components of gangliogliomas (*18*), but in that case they will add little new information.

GFAP is the most commonly used and by far the most useful to date of the immunohistochemical markers for CNS tumors (*18*). It is found in all variants of astrocytoma, in astroblastomas, ependymomas, subependymomas, and in the astrocytic cells of mixed gliomas, gangliogliomas, mixed gliomas and sarcomas, glioblastomas, medulloblastomas, and pineocytomas (*19*).

The other IF proteins, although not exclusive to the nervous system, may be useful in certain circumstances. Metastatic carcinomas may be detected or confirmed by the demonstration of cytokeratin(s). Vimentin is pres-

ent in lymphoma, melanoma, nonmuscle sarcoma, meningioma, schwannoma, and fibrous histiocytoma cells, as well as some ependymomas and anaplastic astrocytomas. Desmin is found in rhabdomyosarcomas and leiomyosarcomas (*18,20*).

A less reliable, but occasionally useful, marker for CNS tumors is the S-100 protein. It has been demonstrated in glial and meningeal tumors, but it has also been found in a variety of unrelated extraneural tissues (*21*). Its main use in neuro-oncology appears to be in the recognition of amelanotic melanoma and of Schwann cell tumors.

Neuron-specific enolase (NSE) is an isozyme of the glycolytic enzyme enolase which in normal conditions is specifically localized in the cytoplasm of neurons and neuro-endocrine cells. It can, however, be demonstrated in neoplastic cells of various other tissues, including cerebral tumors such as glioblastoma, astrocytoma, oligodendroglioma, ependymoma, medulloblastoma, pineocytoma, meningioma, and choroid plexus papilloma (*22*). By electron microscopic immunocytochemistry, all brain tumors examined (primary and metastatic) have exhibited membrane staining for NSE, and many glioma cells have positively stained intracellular filaments. NSE could not be demonstrated on membranes or filaments of normal brain cells.

A useful glycolipid marker for oligodendrogliomas could be galactocerebroside (*23*). The application of myelin basic protein (MBP) for this purpose has so far not been successful (*18*).

In addition, there are a number of potentially useful antigens for brain tumor diagnosis. These include synaptin, D1, D2, D3, Ran-1, α-2-glycoprotein, thy-1, HNK-1, and some unnamed antigenic markers, whose antisera selectively bind to neural elements. Full characterization of these antisera and a study of their specificity for both normal and neoplastic brain tissue cells is essential prior to their use for diagnostic purposes.

## Monoclonal and Polyclonal Sera

A number of normal brain and nervous system antigens have been identified by several investigators using heterologous antisera raised against whole brain, certain areas of the brain, proteins isolated from brain tissue or the retina (*24,25*). Among the best characterized are GFAP, MBP, NF proteins, NSE, and S-100 protein. GFAP, present only in astrocytes and in no other neural or non-neural cell in the CNS, has found wide spread use as a reliable marker aiding in the diagnosis of gliomas. Galactocerebroside, the major glycolipid in myelin, has been shown to be a specific cell surface marker for cultured oligodendrocytes (*24*). Rabbit antigalactocerebroside sera bound specifically to cultured oligodendrocytes and this activity could be completely removed by absorption with myelin or oligodendroglia. Gradient-purified cell populations enriched for human, rat, and lamb neurons or oligodendroglia has been used to raise rabbit antisera (*26*). Extensive cross-absorptions coupled with an indirect immunofluorescence analysis revealed the presence of cell type-specific surface membrane antigens. Antineuronal sera reacted predominantly with neuronal cells but not with other cell types. The anti-oligodendroglia sera were, likewise, demonstrated to be specific, although none of the antisera reacted with purified myelin. Stallcup and Cohn (*27*) raised rabbit antisera against two neuronal and two glial tumors induced by ethylnitrosourea (ENU) in rats. Cross-absorption analyses revealed subsets of specific surface antigens on rat neuronal cells (N1, N2, N3) and glial cells (G1, G2). Using an antiserum raised against a methylcholanthrene-induced mouse glioma, Schachner (*25*) identified a brain-specific antigen, NS- 1, that was enriched in adult white matter but reduced in a myelin-deficient mutant strain of mice. The NS-1 antigen was expressed more in glial, but not in neuron, enriched cell fractions and was found only on glial tumor cells and not on those of neuronal origin. Clearly, heterologous antisera can be produced that will detect antigens unique to various cell types found in normal neural tissue. All of these sera, however, require extensive cross-absorptions that result in lower titers of antibody of functional, not absolute, specificity. We have produced and partially characterized a panel of mouse hybridoma cell lines secreting monoclonal antibodies to bovine S-100 protein. S-100 has been demonstrated in astrocytes within the central nervous system and in Schwann cell and satellite cells of the dorsal root and autonomic ganglia within the peripheral nervous system. Six of these antibodies have been used to demonstrate patterns of distribution of S-100 protein in formalin-fixed, paraffin-embedded sections of rat, mouse, bovine, and human normal brain tissue by immunohistochemical staining by the avidin-biotin immunoperoxidase technique. These antibodies do not react with the calcium-binding proteins, calmodulin or parvalbumin, but do react with a copper-binding protein, neurocuperin. S-100 has been consistently demonstrated by immunohistochemical staining in human neoplasms of astrocytic, Schwann cell, and melanocytic origin and we have used some of these monoclones to demonstrate S-100 in human neuroglial tumors (schwannomas, neurofibromas and astrocytomas) as well as in Schwann cells and Langerhans' cells in human skin.

# Morphology and Classification of Brain Tumors in Man and Animals

## Man

A major objective of any classification of neoplasia is to provide an estimate of biologic behavior and patient prognosis. Therefore, although morphology must constitute the cornerstone of tumor classification, other factors such as patient age, tumor location, involvement of vital structures, intracranial mass effect, and tumor evolution must also be considered. Tumor evolution and progression are especially important: neoplastic transformation may occur at different stages of neuroepithe-

lial cell differentiation and subsequent tumor evolution may result in multiple cell populations that are phenotypically heterogeneous.

The classification of human brain tumors developed by the World Health Organization (WHO) is based primarily on cytological and histological characteristics (*28*). Tumors are divided into those arising from neuroepithelial, nerve sheath, meningeal, lymphocytic, vascular, germ cell, or malformative tissues. Neuroepithelial tumors are subdivided into astrocytic, oligodendroglial, ependymal, pineal, neuronal, and undifferentiated (embryonal) tumors.

Astrocytomas may be classified as fibrillary, protoplasmic, gemistocytic, or pilocytic according to the morphology of the predominant cell type. The cystic and solid cerebellar astrocytoma, the pilocytic astrocytoma of juvenile type, and the optic nerve glioma contain a high proportion of pilocytic astrocytes. These neoplasms may be associated with vascular proliferation and invasion of the leptomeninges, but they are slow-growing and possess a low potential for evolution into an anaplastic tumor. In contrast, the other types of astrocytoma exhibit more diffuse infiltration of the surrounding brain and frequently evolve into an anaplastic astrocytoma or glioblastoma. Anaplasia is characterized by high cell density, cellular and nuclear pleomorphism, nuclear hyperchromasia, frequent mitoses, necrosis, and vascular-mesenchymal proliferation.

The cells in oligodendrogliomas have round to oval nuclei, clear cytoplasm and distinct cell boundaries. There is typically a fine vascular stroma separating clusters of tumor cells. Ependymomas are also composed of uniform cells with round to oval nuclei. Rosettes, canals, and perivascular pseudorosettes are characteristic. Anaplastic oligodendrogliomas and ependymomas may be recognized, but as the tumors evolve, evidence of oligodendroglial or ependymal differentiation may be lost.

Undifferentiated (embryonal) tumors include the glioblastoma, medulloblastoma, medulloepithelioma, primitive polar spongioblastoma, and diffuse glioma. The glioblastoma is an anaplastic neoplasm that exhibits marked cellular and nuclear pleomorphism, necrosis, and mesenchymal proliferation. Most glioblastomas exhibit some degree of astrocytic differentiation, as evidenced by the expression of GFAP. Mixed glioma-sarcomas are thought to arise from the secondary neoplastic transformation of hyperplastic mesenchymal elements in a pre-existing glioblastoma. The medulloblastoma is a cerebellar neoplasm typically composed of undifferentiated neuroepithelial cells. Some medulloblastomas, however, exhibit neuroblastic or neuroglial differentiation. In diffuse gliomatosis, the neoplastic cells exhibit such diffuse and extensive infiltration of the brain that it is difficult or impossible to identify a focal origin for the neoplastic process. The degree of anaplasia is variable.

Tumors of nerve sheath origin are the neurilemmoma (schwannoma, neurinoma) and the neurofibroma. The neurilemmoma exhibits a mixture of compact (Antoni A) and spongy (Antoni B) areas. The neurofibroma is characterized by irregular fascicles of Schwann cells and fibroblasts.

Meningiomas are regarded as tumors arising from cellular elements of the meninges. This view has resulted in the inclusion of the hemangiopericytoma, a vascular neoplasm, as a variant of meningioma. Other variants include the meningotheliomatous, transitional, fibroblastic, psammomatous, and angiomatous meningiomas, which are derived from arachnoidal cells. Hemangiopericytic and papillary meningiomas exhibit more aggressive biologic behavior than other types. The hemangioblastoma is a vascular neoplasm that occurs mainly in the cerebellum, medulla, and spinal cord.

Primary lymphomas and several malformative brain tumors are also recognized, including the craniopharyngioma, epidermoid and dermoid cysts, colloid cyst, and others. Neoplasm may also involve the brain by direct extension from adjacent tissues or by hematogenous metastasis.

## Domestic Animals

Brain tumors have been reported in all species of domestic animals. As mentioned before, the most significant incidence has been observed and reported in the dog and cat. This report is based on primary tumors personally observed in the dog with comments on others species when applicable. Our classification is based on our interpretation of the predominate tumor cell type and the site within the cranial cavity. Neuroglial tumors were observed in 215 dogs. The breed incidence is significant as 147 were in brachiocephalic breeds (boxer 104, Boston terrier 39, bull mastiff 2, English bull 1, and pug 1). Age incidence was from 3 to 15 years with 160 between 6 and 11 years. The male/female ratio was 1.7. The tumors included 118 benign and malignant astrocytomas which were located in the cerebrum (80), brainstem (20), cerebellum (17), and 1 originated in the posterior pituitary. Four dogs had diffuse tumors of the brain which were diagnosed as spongioblastoma or embryonal glioma. There were 60 benign and malignant oligodendrogliomas which were located in the cerebrum (54) and brainstem (6). There were 25 dogs with glioblastoma multiforme located in the cerebrum (24) and in the brainstem (1). Four ependymomas were noted in the third ventricle (3) and cerebellum (1). Four dogs had two or more primary tumors.

Twenty-five animals had tumors of the choroid plexus. They occurred in 15 breeds and in 16 males and 9 females. The age range was from 2 to 11 years with 19 between 5 and 10 years. The site of origin was fourth ventricle (13), lateral ventricle (4), third and lateral ventricle (4), and third ventricle (3). In one animal a gross tumor was not apparent. Microscopically there was meningeal carcinomatosis with carcinoma in situ present in the plexus of the fourth ventricle. Two animals were presented with pelvic limb paralysis which was secondary to CSF metastasis with infiltration of the lumbo-sacral spinal cord and nerve roots.

Tumors of neuronal origin are rare in the dog. Two medulloblastomas and one ganglioglioma were observed.

Tumors of the meninges are common in the dog and cat. Meningiomas were observed in 106 dogs (25 breeds). The age incidence was from 2 and 15 years with 62 between 6 and 12. The male/female ratio of 0.6 simulated the human situation. The site was intracranial (88), intraspinal (15) and intraorbital (3). The intracranial meningiomas in the dog, while histologically similar to man, manifested some significant variations. These included a significant number of basal and plague meningiomas involving the floor of the cranial cavity, a marked incidence of brain infiltration via Virchow-Robins space, the frequent occurrence of focal necrosis with suppuration and while occasionally observed, psammoma bodies were not prominent. Electron microscopy illustrated interdigitations of cellular processes, desmosomes, and tonofilaments. Meningeal sarcomas were observed in 3 dogs.

Tumors of the cranial, spinal and peripheral nerves were common in the dog. Sixty schwannomas were observed which involved the cranial (4) and spinal (39) and peripheral (17) nerves in 23 breeds of dogs. The age incidence was from 2 to 17 years with 43 between 5 and 12 years. The male/female ratio was 2.3. There were 39 involving the spinal cord originating from the roots or brachial plexus and sciatic nerves. Not included in this series are 4 animals with intraparenchymal tumors which were believe to be schwannomas (midbrain 1, spinal cord 3).

## Laboratory Animals

***Mice.*** Swenberg (*4*) reviewed the literature concerning neoplasms of the murine nervous system and concluded that spontaneous brain tumors in mice were extremely rare. There are a number of reports of spontaneous and experimentally induced brain tumors in mice, some of which present detailed descriptions of their morphologic characteristics. However, very few publications present a review of the morphologic characteristics of a range of neoplasms, and address the question of their classification. Ward and Rice (*29*) reviewed brain tumors from a large group of rats and mice, and proposed a simplified histogenetic classification scheme. Morgan (*30*), examined brain tumors found in 42 mice, from a study population of 77,410 mice at the National Center for Toxicologic Research (NCTR), and recommended the use of the new WHO classification (*28*).

Brain tumors from a large population of mice, derived from the National Toxicology Program (NTP archives), were recently examined and classified, and the data combined with that previously reported by Morgan et al. (*30*). This study was based upon hematoxylin and eosin stained sections from both treated and untreated animals. Many of the tumors showed morphologic characteristics reported by other workers for human brain tumors (*19,28*), and were readily classified in the new WHO scheme (*31*). Others were more difficult to classify due to inadequate samples or unusual morphologic features. A total of 17 lipomas were found, the majority of which were in or adjacent to the midline. These tumors were considered to be hamartomas rather than true neoplasms. A total of 59 neoplasms, other than lipomas, were found in the combined slide sets (NTP and NCTR), of which over half were either oligodendrogliomas (27%) or meningiomas (31%). Other neoplasms diagnosed included medulloblastoma, glioblastoma, mixed glioma, ganglioglioma, choroid plexus tumor, angiosarcoma, teratoma, and granular cell tumor. It has yet to be established whether a common system of classification is appropriate for brain tumors of 'mice and men'.

***Rats.*** The need for a histological classification of brain tumors in rats became apparent in the early fifties when research began to focus on the chemical induction of brain neoplasms in this animal species (*32*). Unfortunately, different classification schemes were introduced by various research groups. They varied from distinguishing neuroepithelial tumor types in isomorphic and pleomorphic subtypes including a grading system for the degree of malignancy to ones that simply categorized all neuroepithelial neoplasms as gliomas. The present situation is not much different, which is mainly due to the fact that criteria for diagnosing brain tumors in rodents are based on the general structure of the neoplasm rather than on the cytological features. The current availability of various nervous system markers may lead to a more accurate classification scheme or brain tumors in rodents in the near future.

Two tumor types occurring in rats are still in dispute, i.e., the granular cell tumor and malignant reticulosis.

The granular cell tumor is classified by some as a meningioma and regarded by others to be a separate entity. The histogenesis is still unknown (*33,34*). The tumor is characterized by either sheets or nests of closely packed large, round or oval cells with abundant eosinophilic cytoplasm and an eccentrically placed, often pleomorphic nucleus and a small nucleolus. The two most characteristic features are the abundance of small, discrete, lightly eosinophilic cytoplasmic granules that are PAS-positive and diastase-resistent and the presence of contact with the leptomeninges in nearly 100% of the cases (Fig. 1). A morphologically similar tumor occurs in man, but it is found most often in superficial soft tissues or in the tongue and only incidentally in the central nervous system. The cell of origin have been considered to be the embryonic striated muscle cell, fibroblast, histiocyte, Schwann cell, astrocyte, and a undifferentiated mesenchymal cell. The conclusion of a recent immunohistochemical and ultrastructural study dealing with human granular cell tumors was that the granular cell may be derived from uncommitted possibly nerve-related mesenchymal cell. Recently, we performed an immunohistochemical study on rat granular cell tumors using S-100 protein and GFAP as markers. The granular cell did not stain with either marker sug-

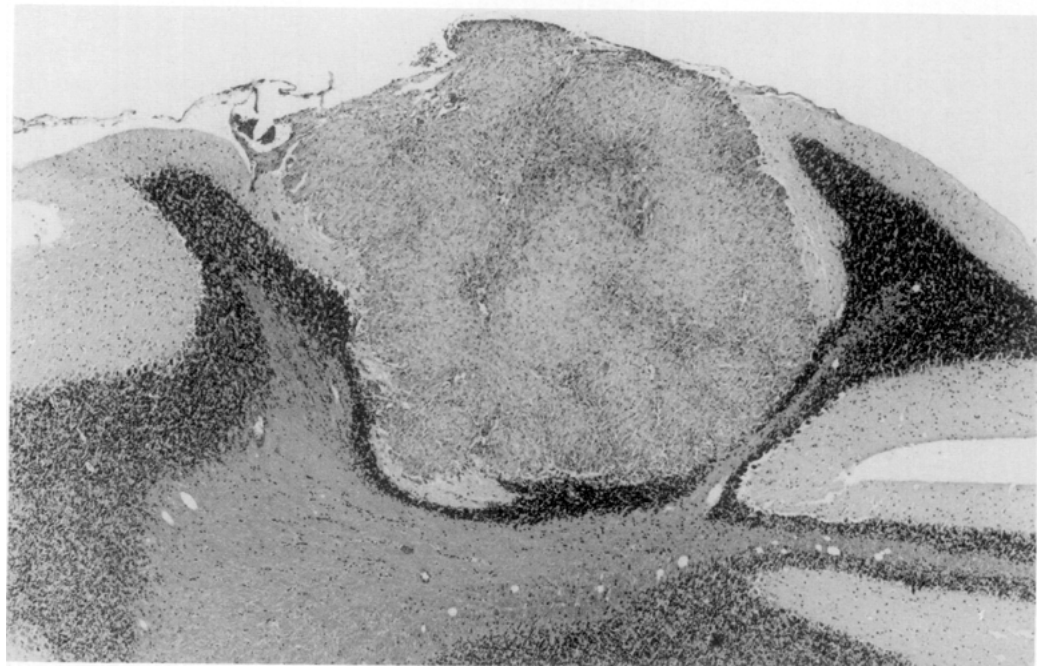

FIGURE 1. Granular cell tumor in the cerebellum of a rat. Note the contact with the meninges. Hematoxylin-phloxine-saffron, ×34.

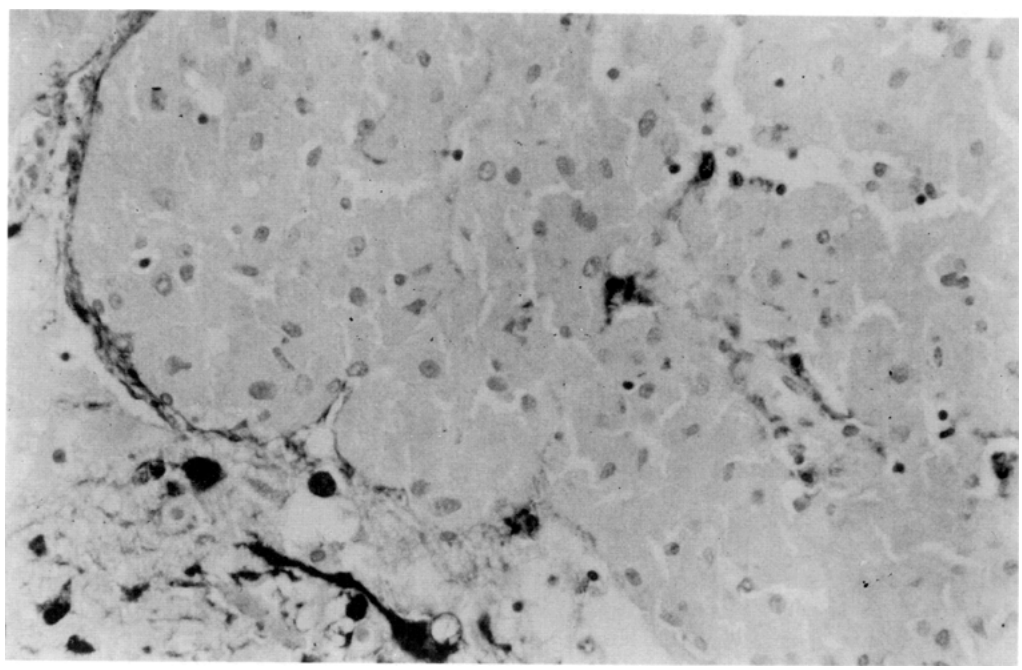

FIGURE 2. Immunoperoxidase staining for S-100 protein. Negative staining of a granular cell tumor. Note positive staining of reactive astrocytes at the margin of the tumor. ×357.

gesting that the Schwann cell (Fig. 2) or the astrocyte is not the cell of origin.

Another tumor type occurring in rats that is in dispute is "malignant reticulosis." Krinke (*35*) found recently that this tumor type was more common than glial neoplasms in Sprague-Dawley derived rats. This conclusion was based on the use of special stains including immunohistochemistry, although they did not use anti-Ig antibodies. They suggested that a number of tumors diagnosed as glial neoplasms in rats are probably cases

of reticulosis. The preliminary results of an immunohistochemical study performed by us cannot exclude this possibility since a number of tumors that were diagnosed as astrocytomas were negative for GFAP (Figs. 3 and 4). Further studies, including the use of anti-Ig antibodies, are necessary to substantiate this suggestion.

## Differentiation, Transformation and Progression of Neuroglia Cells

### Differentiation

Neoplastic disease essentially implicated populations of cells of renewal, i.e., stem cells that lack the differentiated characteristics of the tissue to which they give rise but have the potential for the expression of these characteristics and transmit this potential to their progeny. The potential is only fully realized in the postmitotic cells in the terminal stage of differentiation. Cell replication is a prerequisite for malignant transformation. When the undifferentiated stem cell is the target of transformation, it is converted into a neoplastic stem cell which to a variable extent has a capacity for divergent differentiation similar to that of the normal stem cell that has not been so transformed. In CNS tumors this is illustrated by the group of central embryonal neuroepithelial neoplasms, in which a correlation can be drawn between the various tumor types and the sequential stages of neurocytogenesis occurring in the forebrain.

A reserve population of stem cells capable of further differentiation in their progeny is present in all tissues and organs actively involved in regular tissue renewal. In the mammalian brain, as repeatedly shown in experimental neurooncogenesis, a highly susceptible target of neoplastic transformation are the migrating subependymal cells, which are still cycling at a relatively late stage of central neuroepithelial development, i.e., shortly before and after birth, and which are the glial cell precursors whose progeny will later differentiate into astrocytes and oligodendrocytes. One possible extrapolation is that in human gliomas fetal neuroepithelial cells in the late stages of pregnancy might be a selected target for subsequent neoplastic development. This would account for the relatively high incidence of gliomas in childhood and is supported by a statistical study of children with CNS tumors, in whom age at diagnosis has been correlated with the risk of later recurrence.

The reserve neuroepithelial cell population in the adult human brain is low, but under the appropriate stimulus astrocytes in $G_0$ phase are capable of reentering the cycle and resuming proliferation. The main loci of continuing neurocytogenesis that may persist in postnatal life may comprise the subependymal glial cell layer; the "myelination glia" at the time of myelinogenesis; the fetal external granular layer of the cerebellum; the dentate fascia of the hippocampus; and, perhaps, the subpial granular layer of the cerebral hemispheres.

The nature, stage of differentiation and differentiating potential of the CNS cells targetted by the first "hit" of neoplastic tranformation will determine the type of tumor that will develop and the differentiating potential of its cellular components. It must, however, be re-

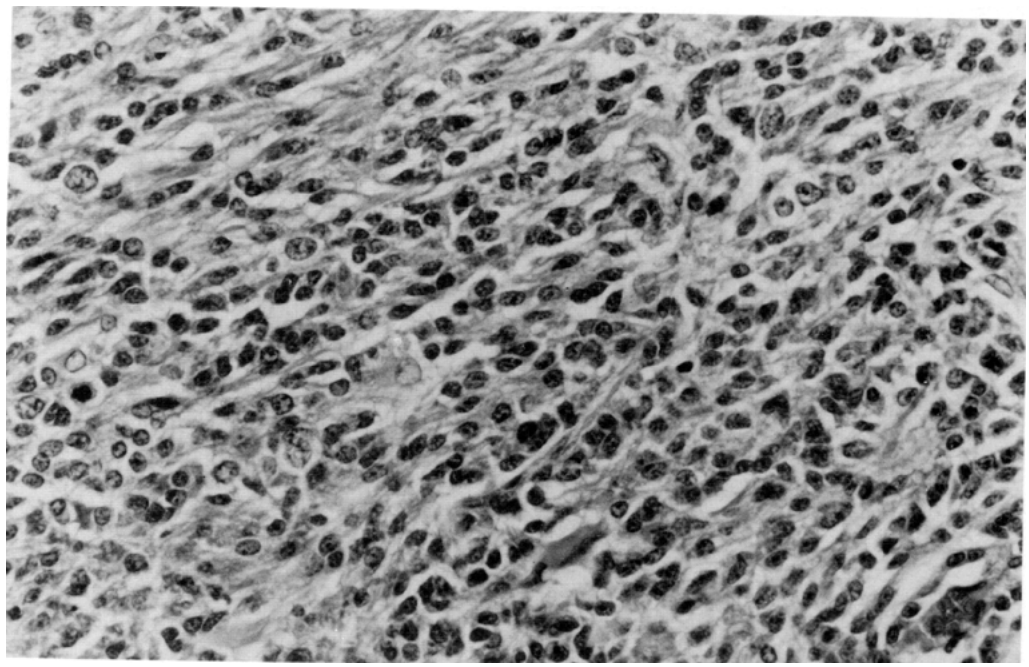

FIGURE 3. Astrocytoma in the cerebrum of a rat. Nuclear pleomorphism and mitoses are present. Hematoxylin-phloxine-saffron, ×357.

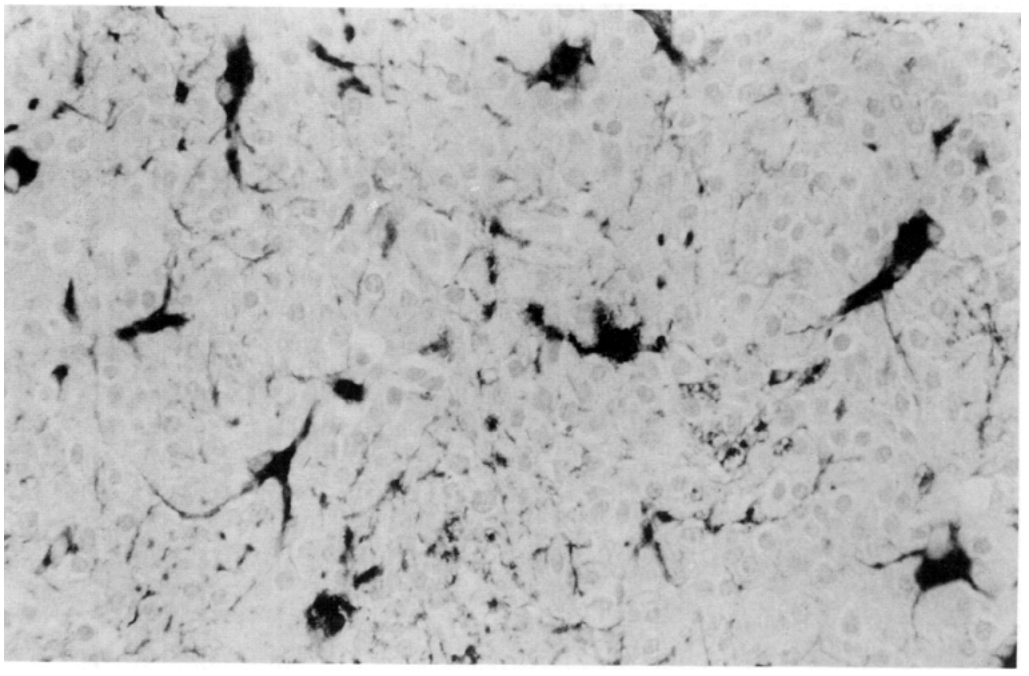

FIGURE 4. Immunoperoxidase staining for GFAP. Negative staining of the neoplastic astrocytes whereas reactive astrocytes stain intensely. ×357.

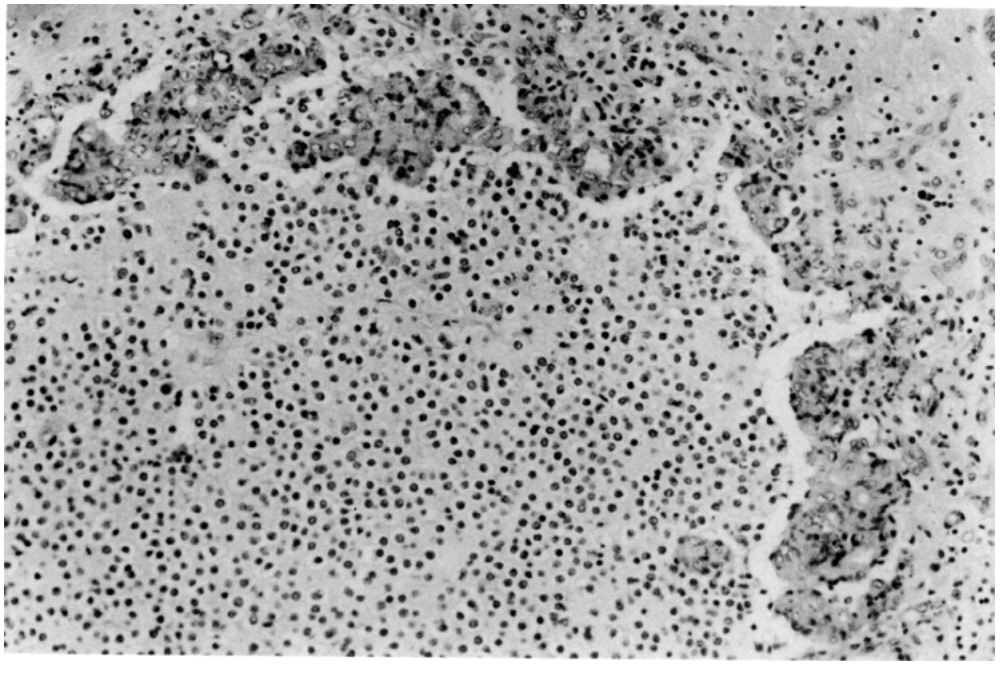

FIGURE 5. Rat oligodendroglioma showing the characteristic honeycomb pattern and prominent vascular proliferation at the margin of the lesion. (Hematoxylin-phloxine-saffron, ×147.

membered that during the latent period of carcinogenesis, displacement of the targeted cells and their progeny is likely to have occurred. Therefore, a glial precursor cell "hit" in the course of its migration from the subependymal zone could be the source of a cortical glioma in later life. In the experimental cerebellar medulloblastomas induced in hamsters by the JC strain of human polyoma virus, the transformed cells are presumed to be those of the external granular layer, which retain their mitotic activity in the course of their inward migration: the tumors themselves appear to originate from the internal granular layer.

Divergent differentiation in central neuroepithelial tumors is most often expressed in embryonal neoplasms, such as the medulloepithelioma, the cerebellar medulloblastoma, and the pineoblastoma. In differentiated, relatively mature neoplasms, as in gangliogliomas, it is inferred to have resulted from the differentiation of primitive bipotential precursor cells. Curious examples illustrating the ontogenetic memory expressed by the differentiation process are provided by some pineoblastomas (fleurettes, indicating retinoblastic differentiation) and by choroid plexus papillomas (GFAP expression).

Heterogeneity of malignant tumor cell populations, long recognized as "anaplasia," is currently being emphasized not only as a phenotypic feature, but also as an expression of genotypic (karyotypic) variability. Genetic lability increases as the tumor becomes more malignant and is ascribed to the progressive increase in mutation rates to which the resulting subpopulations are subjected. The variability of GFAP expression in tumor cell lines obtained from human gliomas and transplanted in athymic mice is consistent with the development of subpopulations with different genetic properties.

There is considerable evidence that human malignant disease is most often of monoclonal origin. Monoclonality could be reconciled with the occasionally demonstrated multicentricity of malignant gliomas and with the existence of extremely diffuse forms (gliomatosis cerebri) by assuming that, as the process of transformation is a progressive multistep event, the first mutational "hit" might involve a single clone of neuroepithelial cells in the course of their migration from the primitive ventricular zone: after a varying latent period, the subsequent hit or hits necessary to promote tumor formation would involve the multiple and distantly separated progeny of the originally targeted neuroepithelial cell(s). A new kinetic round would be essential before the phenotypic development of neoplasia.

Along a similar line of reasoning, divergent differentiation with the development of a mixed glioma composed of well-differentiated cells could still be compatible with monoclonality if the last mutational event involved cells in the preterminal stage of differentiation that were the progeny of an originally targeted precursor cell.

Neoplastic cell populations exhibit a dynamic state of flux between differentiation and anaplasia. Cellular differentiation is presumably determined by the ontogenetic memory of the cell clone originally hit, and is associated with a decycling of the proliferating populations. Anaplasia, on the other hand, results from the recruitment of proliferating cells from the nonproliferating pool, and the progressive selection of clonogenic subpopulations (*36*).

## Progression

In man the early stages of neoplastic transformation in the nervous system are poorly characterized (*37*). This is in contrast to experimental animals where sequence studies of carcinogen-exposed brains have shown how clusters of proliferating glial cells are followed by development of microtumors and thereafter larger, invasive tumors. So far, such studies on intact brains have not enabled a closer examination of phenotypic changes at the cellular level.

In order to overcome this problem, an *in vivo–in vitro* system for the study of malignant transformation and progression of fetal rat brain cells has been developed (*38*). Pregnant BD IX rats were given a single IV injection of 75 μg/g ENU at the 18th day of gestation. One day afterwards the transplacentally exposed fetal brains were transferred to long-term cell culture. The cells underwent a characteristic sequence of phenotypic alterations ending with tumorigenic properties after 200 days in culture, i.e., the same period as it takes to produce CNS tumors in ENU-exposed animals *in vivo*. A survey of this sequence of malignant transformation is given and discussed in relation to available data from the development of intracranial tumors.

During the first 2 weeks of culture, ENU-exposed brain cells had the same morphological appearance as untreated control cultures although there was an increased outgrowth of differentiated neuroglial cells (oligodendrocytes, astrocytes, and neurons). The earliest sign of morphological cell changes was after 3 weeks when neuroglial cells exhibited large, dorsal ruffling membranes, not oriented in the direction of movement.

After 60 days neuroglial cells grew in characteristic organoid nodules consisting of astrocytes, oligodendrocytes and neurons extending to the periphery. At this stage control cultures only consisted of flat, epithelioid neuroectodermal cells.

The next stage was after 100 days with the onset of rapid proliferation of morphologically "transformed" cells with atypical nuclei, criss-cross growth pattern and formation of pile-up foci. At the same time a more stable phenotype with expression of mainly astrocytic markers resulted. These cells were not tumorigenic by reimplantation into newborn rats. After 140 days loss of anchorage dependence was observed as assayed by the formation of agar colonies. Finally, after 2 to 300 days the cells were biologically malignant as shown by the formation of malignant, gliomalike tumors after reimplantation into isogeneic host animals. At the same time

the cells showed invasion by confrontation with fetal rat brain tissue in organ culture (*39*).

The resulting malignant cells contained glia-characteristic markers as GFAP and S-100 protein, but in addition exhibited NSE activity. The latter finding indicates that ENU is acting on multipotent neuroectodermal cells.

In conclusion, malignant transformation of fetal rat brain cells in monolayer culture is accompanied by early morphological changes ultimately resulting in invasiveness and tumorigenicity.

## Chromosomal Abnormalities

Our overall goal is to define the chromosomal abnormalities of malignant human gliomas and to determine the theoretical and practical significance of these changes. We have karyotyped 25 malignant human gliomas including 3 anaplastic astrocytomas (AA), 21 glioblastoma multiforme (GBM), and 1 gliosarcoma (GS) in direct preparation, short-term culture or both. Six of these tumors (3 GBM) are near-tetraploid (group II) and contain double minutes (DMs). Two of these tumors are lacking two copies of No. 22; in the third, the chromosomes are too short and poorly banded for specific analysis. The remaining 16 tumors (1 AA, 14 GBM, 1 GS) have near diploid stemlines (group III). Eleven of these sixteen tumors have gains of normal or abnormal No. 7, 11 of them have losses of No. 10 and 10 have both of these changes. Ten tumors have loss (*3*) or structural rearrangements (*7*) of No. 9. In all seven cases the breakpoint is at the centromere or in the *p* arm. Ten group III tumors contain DMs.

Clinical and histologic parameters of these cases were examined to determine if they could predict the karyotype or if it is an independent variable. The ratio of males to females was approximately the same within each group as it was for the overall series. All three patients less than 40 years old were in group I but there was otherwise no difference in age among the groups. Group II tumors were more likely to have abundant multinucleated giant cells, but not other histologic feature characterized any of the groups.

One explanation for the different karyotypic groups is that they are related through progression. The karyotypes of three group III tumors which established in culture showed the same pattern of evolution consisting of doubling of the stemline or a closely related population followed by acquisition of new markers; one line changed again to near-pentaploid. These studies suggest that group II tumors are at a later stage of karyotypic progression than groups I and III. Long-term studies are in progress to determine if patients with group II tumors have a worse prognosis.

Among the other explanations for the different karyotypic groups of malignant human gliomas is the possibility that these specific chromosomal changes have resulted in increased expression of particular oncogenes. For example, ErbB is located on chromosome No. 7. Tumors with extra copies of No. 7 may be carrying multiple copies of activated ErbB. The Sis oncogene is located on No. 22, and Abl on No. 9, but on the *q* arm. It is possible that a previously undiscovered oncogene is located on 9*p* and on 10. Losses of Nos. 9, 10, or 22 could allow expression of an altered oncogene on the remaining chromosome. Since many of the tumors with chromosomal loss also contain DMs, these bodies may contain amplified oncogenes. As human gliomas are examined for their expression of oncogenes, the role of specific chromosomal abnormalities of malignant human gliomas may be clarified.

Another explanation for the differing karyotypic pattern of human gliomas is that they may be produced by different inducing agents. Both leukemia and sarcomas induced in rats by DMBA contain gains and structural abnormalities of No. 2 while Rous sarcoma virus-induced rat sarcomas contain extra copies of No. 7. The only similar data available for brain tumors show consistent gains of No. 4 in cell lines derived from ENU-induced rat brain tumors. We have begun a comparative study of karyotypes of brain tumor induced in F344 rats with avian sarcoma virus (ASV), ENU and acrylonitrile (AN). To date, six AN tumors have been evaluated—2 have normal stemlines with occasional variant cells; four contained no spreads for evaluation. As these studies are completed we will be able to determine if different inducing agents produce different karyotypic patterns among rat gliomas.

In summary, specific chromosomal abnormalities characterize groups of human gliomas. These patterns cannot be predicted using clinical or histologic parameters. Chromosomal composition may be useful in predicting prognosis or responsiveness to specific therapeutic agents. Alternatively, it may define groups of gliomas produced by different inducing agents or may identify tumors which carry the same activated oncogene.

# Experimentally Induced Brain Tumors

## Chemically Induced

Brain tumors can be induced in experimental animals by a broad series of chemical carcinogens, ranging from large polycyclic hydrocarbons to small alkylating agents. The first experimental brain tumors were induced by directly implanting pellets of methylcholanthrene into the brains of mice. This method was extensively used for nearly 30 years, employing a variety of species. Tumors arise in tissue adjacent to the pellet, with the predominant tumor type depending on the site of implantation. The primary advantage of this method is the localization of the tumors.

In contrast to the polycyclic hydrocarbons, a new group of chemicals that induced a high incidence of brain tumors following systemic exposure was identified by Druckrey (*40*) and co-workers in the 1960s. Best studied among these are MNU and ENU. Detailed investigations on the morphology, dose-response, pathogenesis

and mechanisms of induction of brain tumors induced by this family of carcinogens have been completed.

Only a small number of the other chemicals that have been tested for carcinogenicity have induced brain tumors in experimental animals. Oral or inhalation exposure of rats to acrylonitrile produced dose-related increases in brain tumors tentatively classified as anaplastic astrocytomas. A low, but dose-related increase in tumors having a similar morphology has been reported following inhalation exposure of rats to ethylene oxide (*41*).

While gliomas have been induced by all of the chemicals listed above, it is of interest that no chemical has been shown to cause granular cell tumors.

## Virally Induced

Viruses have been important agents for experimental brain tumor induction since the first experiments in 1936 by Va'squez-Lo'pez (*42*) who showed that sarcomas could be induced in the brains of chickens by intracerebral inoculation of Rous sarcoma virus. Despite the importance of oncogenic viruses as agents for experimental brain tumor induction spontaneous brain tumors in either animals or humans have not been shown to be caused by viruses. Endogenous retrovirus has been demonstrated in a number of chemically induced intracerebral tumors but the significance of that retrovirus with regard to playing a role in transformation remains to be demonstrated. In contrast to chemically induced brain tumors there is a reasonably clear-cut association with specific classes of viruses with the type of brain tumors induced. The morphology of many of the brain tumors induced following intracerebral inoculation of virus is quite distinctive (*43–45*).

Most viruses must be inoculated intracerebrally in high doses in neonatal animals for successful brain tumor induction to occur. Nevertheless, Avian sarcoma virus (ASV) and human papovavirus will induce brain tumors following adult inoculation. The majority of viruses that induce brain tumors have been inoculated across species barriers from the virus species of origin, and thus are in nonpermissive systems. No viral replication occurs although virus genome and virus-related gene products are in the transformed brain tumor cells. Some retroviruses (such as Simian sarcoma virus (SSV) in primates and Murine sarcoma virus (MSV) in rodents) have been inoculated intracerebrally in permissive hosts and virus replication and full genome expression occurs.

The most widely used brain tumor models are the ASV-induced rat anaplastic astrocytoma for therapeutic and tumor biological studies, and the large animal model human papovavirus-primate and ASV-canine model for imaging and diagnosis.

Table 3 summarizes the range of tumor types, viruses and predominant hosts for experimental brain tumor induction.

## Radiation Induced

A group of rhesus monkeys is being observed in a lifetime study of the delayed effects of total body proton irradiation. The animals were each given a single dose of one of five selected energies of protons covering the spectrum of energies and doses to which an unprotected astronaut might be exposed in space during a major solar flare event. An unusual incidence of intracranial neoplasms has been observed in a group of 72 rhesus monkeys following exposure to 55 MeV protons. The animals were two years of age when exposed at the Oak Ridge Isochronous Cyclotron during April, 1965. The monkeys were confined in wire mesh cylinders and rotated at 2 rpm in a proton beam perpendicular to the spinal column. The dose rate was approximately 50 rads/min and total doses ranged from 25 to 800 rads. The 55 MeV particle deposits all its energy in the first 2.5 cm of wet tissue, terminating in a "Bragg peak." Rotating the subjects in the beam subjected a large volume of brain tissue to Bragg peak proton irradiation. In the subsequent 19-year period, eight monkeys exposed to 400 to 800 rads have developed similar fatal brain tumors which were classified as glioblastoma multiforme. Characteristic features of this tumor type were abnormal mitoses, vascular proliferation, bizarre giant cells, necrosis, and pseudopallisading of nuclei. Only one naturally occurring glioblastoma has been reported in a rhesus monkey to date. At the present time 30 of the original 72 irradiated animals in the 55 MeV group remain alive, and 8 years have elapsed since the last glioblastoma was diagnosed.

# Conclusion

The increased incidence of brain tumors in man in certain localities has suggested to some that exposure to environmental chemicals has the potential to cause brain tumors in man. We, therefore, felt it was worthwhile to review the brain tumors in the F344 rat and B6C3F1 mouse, which are used as test animals in the National Toxicology Program (NTP). Specifically, we wanted to learn how frequently these tumors occurred spontaneously, whether they could be induced and whether these tumors had any similarity to brain tumors in man.

The incidence of naturally occurring brain tumors was only 1.1% in both male and females F344 rats when the studies were terminated at 2 years. In lifetime studies the incidence was 2.9% and 2.2% in males and females, respectively. The incidence was fairly consistent between studies. The incidence never exceeded 4% (2/50 animals) for studies terminating at 2 years of age. The tumors were even less common in mice (*46*).

Over 100 rat brain tumors were found in the NTP collection. Each case was reviewed by one or more pathologists and over 20 cases were reviewed by participants in this workshop. Since many of these pathologists were familiar with brain tumors in man and domestic animals it was possible to draw some conclusions about the rat lesions. First, we did not recognize any rodent brain tumor as having the morphological pattern of glioblastoma multiforme as it occurs in man. Yet in many cases the individual glia were quite atypical

**Table 3. Specificity of virally induced experimental brain tumors.[a]**

| Experimental brain tumor type | Inducing viruses | | |
|---|---|---|---|
| | Group | Type | Predominant host |
| Anaplastic astrocytoma glioblastoma multiforme[b] | RNA oncornavirus | ASV, MSV, SSV | Rats, dogs, primates |
| | DNA papovavirus | Human JC | |
| | DNA adenovirus | SA-7 | |
| Pilocytic astrocytomas | RNA oncornavirus | ASV | Rats, dogs |
| Gemistocytic astrocytoma | RNA oncornavirus | MSV | Mice |
| Medulloblastoma | DNA papovaviruses | Human JC (Mad-1,-2 stains) | Hamsters |
| | DNA adenoviruses | SA-7, HA-12 | |
| Neuroblastoma or retinoblastoma | DNA adenovirus | HA-12 | Rats |
| | DNA papovaviruses | Human JC | |
| Ependymoma | DNA adenovirus | SA-7 | Hamsters |
| | DNA papovaviruses | SV-40, Human JC | |
| Choroid plexus papilloma | DNA papovaviruses | SV-40, Human BK | Hamsters |
| | DNA adenovirus | CELO | |
| Meningioma | DNA papovaviruses | Human JC<br>Bovine papilloma | Cows |
| Sarcomas | DNA papovaviruses | Human JC<br>Murine polyoma<br>Bovine papilloma | Hamsters, rats |
| | DNA adenoviruses | SA-7, HA-12, HA-18 | |
| | RNA oncornaviruses | ASV, MSV | |
| Pineocytoma | DNA papovavirus (Mad-1,-4 strains) | Human JC | Hamsters |

[a] Modified, from Walker and Bigner (*45*).

[b] Glioblastoma multiforme similar to the spontaneous human neoplasm with pleomorphic cells, endothelial proliferation, necrosis, and pseudopallisading is a rare experimentally induced tumor, even in primates.

and less differentiated than the well differentiated glial tumors in man.

Oligodendroglial tumors were quite common and were characterized by a prominent vascular proliferation often at the margin of the lesion (Fig. 3). Glial tumors were frequently a mixture of astrocytes and oligodendroglia and in these cases vascular proliferation was a usual feature. As in man, some tumors appeared to be as almost pure population of astrocytes. It was felt best to separate the tumors into astrocytomas, mixed oligo-astrocytomas, and oligodendrogliomas. There was no agreement on how to separate these three tumor types but one suggestion was that if a tumor was composed of 80% or more of one cell type it should be given that diagnosis and reserve mixed for tumors where the two cell populations varied between 20 to 80%. In any case, it is important when evaluating a toxicological study to clearly state your criteria and then be consistent throughout the study. The other tumors found in the rat consisted of ependymomas, medulloblastomas, choroid plexus tumors, granular cell tumors and meningiomas. The granular cell tumors were quite characteristic (Fig. 1) and appear to arise in or be associated with the meninges.

An area of concern to toxicologists is focal lesions consisting of an increased number of glial cells. In some studies these have been called gliosis when the lesion was too small to warrant a diagnosis of glioma even though it resembles larger lesions that were diagnosed as gliomas. It was noted that in man gliosis is considered a reaction process and often an inciting factor can be found. In man, small lesions are sometimes diagnosed as incident glioma, especially when there is atypia of the glial cells. With neurocarcinogens, such as ethylnitrosourea, acrylonitrile and avian sarcoma virus, very small microscopically detectable tumors or "pre-neoplastic" cellular proliferations are readily identifiable and quite distinguishable from reactive gliosis. There is no presently known neurocarcinogen which has gliosis as an intermediate transitional step as part of neoplastic progression and transformation. It therefore is prudent to make the diagnosis of gliosis only as a reactive process which usually is associated with a clear structural abnormality such as infarct, abscess, hydrocephalus, or encephalitis.

In man specific cellular markers such as GFAP and S-100 protein have proved useful in the characterization of brain tumors in man. It was hoped that similar results could be obtained in rats. Over 60 rat brain tumors from the National Toxicology Program were examined for the presence of these markers in Dr. Bigner's laboratory at Duke University using immunoperoxidase techniques. The rat brain tumors were uniformly negative for both markers. It is known that tissue fixation and handling can reduce antigenic determinants. All of the material was formalin fixed, often for years. However, in the tumors, reactive astrocytes strained strongly for GFAP (Fig. 4) and S100 protein, suggesting fixation is not the problem. Our current thought is that quantitative expression for these two markers is less in rat than in human brain tumors, and less than in reactive astrocytes of rat.

Ultrastructural studies are of limited value in the rat especially in undifferentiated tumors. If specific ultra-

structural features exist then electron microscopy can be helpful. An example would be differentiating an amelanotic melanoma from a brain tumor. One diagnostic aid that appears exciting is nuclear magnetic resonance imaging. Currently, very clear brain images can be obtained in the rat. This should allow one to detect early lesions and follow the progression of the tumor.

The sensitivity of the F344 rat and the B6C3F1 mouse to detect chemicals capable of causing brain tumors is unknown. More than 300 chemicals tested routinely by NCI/NTP in 2-year studies did not induce brain tumors of mice and only two (propyleneimine and propane sultone) induced brain tumors in rats (*29,47*). In both cases the rats were of the Sprague Dawley stock. Since brain tumors are uncommon in the F344 rat and B6C3F1 mouse plus the fact that the control rates are very constant, even a small chemically associated increase in brain tumors may be quite significant.

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